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Saponin and Sapogenol. XL.[1] Structure of Sophoraflavoside I, a Bisdesmoside of Soyasapogenol B, from Sophorae Radix, the Root of *Sophora flavescens* AITON

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A new bisdesmoside of soyasapogenol B (**3**), named sophoraflavoside I (**2**), was isolated together with soyasaponin I (**1**) from Sophorae Radix (*Sophora flavescens* AITON, root). By employing a photochemical cleavage method for the glucuronide linkage in **2** and on the bases of chemical reactions and spectral analyses, the structure of sophoraflavoside I has been determined to be 3-*O*-[α-L-rhamnopyranosyl(1→2)-β-D-galactopyranosyl(1→2)-β-D-glucuronopyranosyl]-22-*O*-[β-D-glucopyranosyl(1→2)-α-L-arabinopyranosyl]soyasapogenol B (**2**).

Keywords——Sophorae Radix; *Sophora flavescens*; sophoraflavoside I; soyasaponin I; glucuronide linkage selective cleavage; glucuronide linkage photolysis; oleanene-bisdesmoside; oleanene-bisdesmoside ^{13}C-NMR

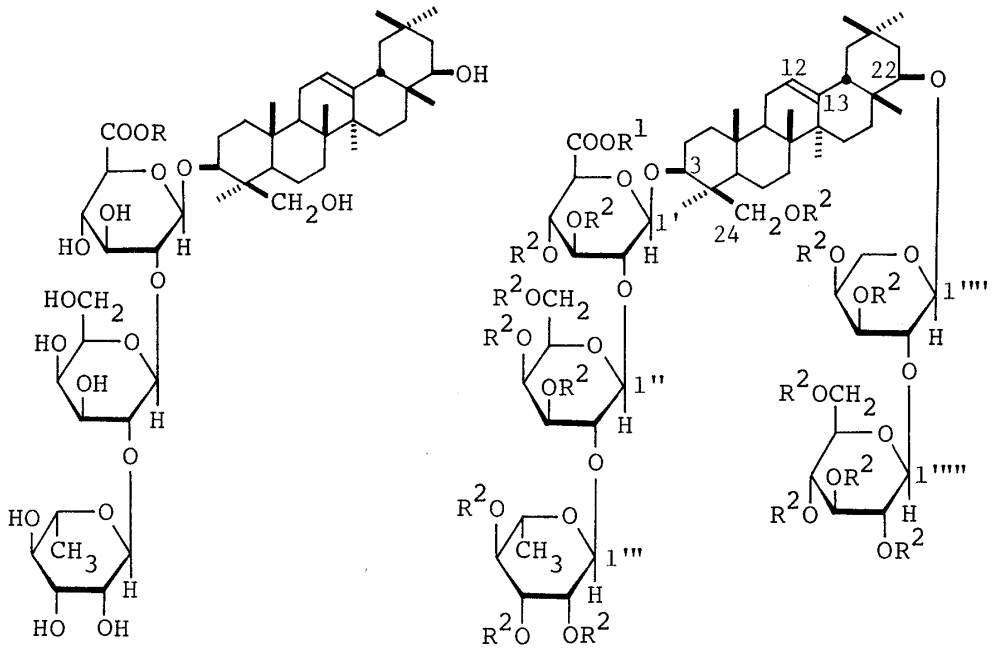
Sophorae Radix, the root of *Sophora flavescens* AITON (Leguminosae), has been prescribed in Chinese Herbal Formulae as an antipyretic, a diuretic, or an anthelmintic. It has also been used as a folk medicine, *e.g.*, as a bitter stomachic, an antiinflammatory drug, an antidiarrheic, or a remedy for dermatosis. In regard to the chemical constituents of Sophorae Radix, alkaloids[2] and flavonoids[3] have so far been characterized. As a part of our continuing chemical studies on the bioactive principles of leguminous naturally occurring drug materials,[1,4,5] we have investigated the glycosidic constituents of Sophorae Radix imported from Korea and have isolated a new bisdesmoside, named sophoraflavoside I (**2**), together with soyasaponin I (**1**).[6] This paper describes the structural elucidation of this new pentaglycoside.[7]

The methanolic extract of the crude drug was partitioned into a 1-butanol–water mixture and the 1-butanol soluble portion was treated with ethyl acetate and methanol to separate the glycosidic fraction. Purification of the glycosidic fraction by a combination of various column chromatographies (ordinary phase and reversed phase) followed by treatment with ionic resin furnished soyasaponin I (**1**) and sophoraflavoside I (**2**).

The infrared (IR) spectrum of sophoraflavoside I (**2**) showed hydroxyl and carboxyl absorption bands. Acidic hydrolysis of **2** gave soyasapogenol B (**3**), whereas methanolysis of **2** with hydrogen chloride and methanol afforded **3** and methyl L-arabinoside, methyl D-galactoside, methyl D-glucoside, methyl D-glucuronide, and methyl L-rhamnoside in equimolar ratio. Diazomethane treatment of **2** effected methylation to provide the monomethyl ester (**2a**).

The carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectrum of **2a** taken in pentadeutero(d_5)-pyridine exhibited signals assignable to five pyranosidic anomeric carbons at δc 98.3 (d, $J_{CH}=162$ Hz, arabinoside C_1), 102.2 (d, $J_{CH}=166$ Hz, galactoside C_1 and rhamnoside C_1), 105.4 (d, $J_{CH}=157$ Hz, glucuronide C_1), and 105.6 (d, $J_{CH}=157$ Hz, glucoside C_1). Thus, the glycosidic configurations in **2a** were suggested to be α-pyranosidic for L-

arabinoside and L-rhamnoside linkages[8] and β-pyranosidic for D-galactoside, D-glucoside, and D-glucuronide linkages.[1,5,9] In addition, detailed comparison of these ^{13}C-NMR data for **2a** with those for soyasaponin I methyl ester (**1a**), soyasapogenol B (**3**), (Table I) and L-arabinopyranosides having a sugar moiety at the C-2[10] led us to presume that **2a** is a bisdesmoside of soyasapogenol B (**3**), which possesses a methylated α-L-rhamnopyranosyl(1→2)-β-D-galactopyranosyl(1→2)-β-D-glucuronopyranosyl residue and a β-D-glucopyranosyl(1→2)-α-L-arabinopyranosyl residue.



1: R=H (soyasaponin I)
1a: R=CH_3

2: $R^1=R^2=H$ (sophoraflavoside I)
2a: $R^1=CH_3$, $R^2=H$
2b: $R^1=R^2=CH_3$

Chart 1

In order to chemically elucidate the structures of carbohydrate moieties in sophoraflavoside I (**2**), we next applied a photochemical cleavage method,[11] which is specified for selective cleavage of the glucuronide linkage in oligoglycosides.[12] Irradiation of a methanolic solution of sophoraflavoside I (**2**) with a 500 W high-pressure mercury lamp furnished a prosapogenol (**4**) and a carbohydrate mixture, and from the latter an anomeric pair of disaccharide heptaacetates (**5a**, **5b**)[13] were characterized after acetylation. The structure of the prosapogenol (**4**) was elucidated in the following manner.

Methanolysis of the prosapogenol (**4**) provided methyl L-arabinoside and methyl D-glucoside in 1 : 1 ratio together with the aglycone soyasapogenol B (**3**). Methylation of **4** with methyl iodide and dimsyl carbanion[14] afforded an octa-*O*-methyl derivative (**4a**), which exhibited signals due to eight methoxyl groups and two anomeric protons in its proton nuclear magnetic resonance (^{1}H-NMR) spectrum. Methanolysis of **4a** provided 3,24-di-*O*-methylsoyasapogenol B (**3a**),[6b] methyl 2,3,4,6-tetra-*O*-methylglucopyranoside (**a**), and methyl 3,4-di-*O*-methylarabinopyranoside (**b**). Consequently, the structure of the prosapogenol (**4**) can be expressed as 22-*O*-[β-D-glucopyranosyl(1→2)-α-L-arabinopyranosyl]soyasapogenol B.

Methylation of sophoraflavoside I (**2**) with methyl iodide and dimsyl carbanion afforded a hexadeca-*O*-methyl derivative (**2b**), which lacked hydroxyl absorption bands but showed ester absorption bands in its IR spectrum. The ^{1}H-NMR spectrum of **2b** exhibited signals due

TABLE I. ^{13}C-NMR Data for Soyasapogenol B (**3**), Soyasaponin I Methyl Ester (**1a**), and Sophoraflavoside I Methyl Ester (**2a**)

		3	**1a**	**2a**
Sapogenol moiety	C-3	80.0	91.3	91.5
	C-12	122.3	122.3	122.8
	C-13	144.7	144.7	144.3
	C-22	75.4	75.5	80.9
	C-24	64.4	63.5	63.0[a]
3-*O*-β-D-Glucurono-pyranosyl moiety	C-1′		105.3	105.4
	C-2′		77.9	77.8
	C-3′		76.3	76.5
	C-4′		72.5	72.8
	C-5′		76.8	77.2
	C-6′		170.0	170.2
	(OCH_3)		(52.0)	(52.1)
2′-*O*-D-Galacto-pyranosyl moiety	C-1″		101.7	102.2
	C-2″		77.5	77.6
	C-3″		74.3	74.4
	C-4″		71.0	71.3
	C-5″		76.8	76.9
	C-6″		61.7	62.1
2″-*O*-α-L-Rhamno-pyranosly moiety	C-1‴		102.0	102.2
	C-2‴		72.1	72.0
	C-3‴		72.5	72.3
	C-4‴		73.3	73.4
	C-5‴		69.2	69.4
	C-6‴		18.7	18.9
22-*O*-α-L-Arabino-pyranosyl moiety	C-1⁗			98.3
	C-2⁗			80.1
	C-3⁗			71.8
	C-4⁗			66.8
	C-5⁗			63.7[a]
2⁗-*O*-β-D-Gluco-pyranosyl moiety	C-1′′′′′			105.6
	C-2′′′′′			75.6
	C-3′′′′′			78.3[b]
	C-4′′′′′			71.8
	C-5′′′′′			78.1[b]
	C-6′′′′′			63.0

25 MHz, in d_5-pyridine, δc.
a, b) Assignments may be interchangeable within the same column.

to sixteen methoxyl groups and five anomeric protons. Lithium aluminum hydride reduction of **2b** and subsequent methanolysis of the product furnished 24-*O*-methylsoyasapogenol B (**3b**), methyl glycosides **a** and **b** (*vide supra*), methyl 2,3,4-tri-*O*-methylrhamnopyranoside (**c**), methyl 3,4,6-tri-*O*-methylgalactopyranoside (**d**), and methyl 3,4-di-*O*-methylglucopyranoside (**e**). Thus, the structure of a trisaccharide moiety in **2**, which is attached to 3β-OH of the aglycone, has been clarified.

The structure of 24-*O*-methylsoyasapogenol B (**3b**) has been confirmed on the following bases. The IR spectrum of **3b** showed the presence of hydroxyl groups, while the ^{1}H-NMR spectrum showed a signal due to one methoxyl group and a two-proton quartet assignable to the 24-methyleneoxy function. The mass spectrum (MS) of **3b** exhibited fragment ion peaks

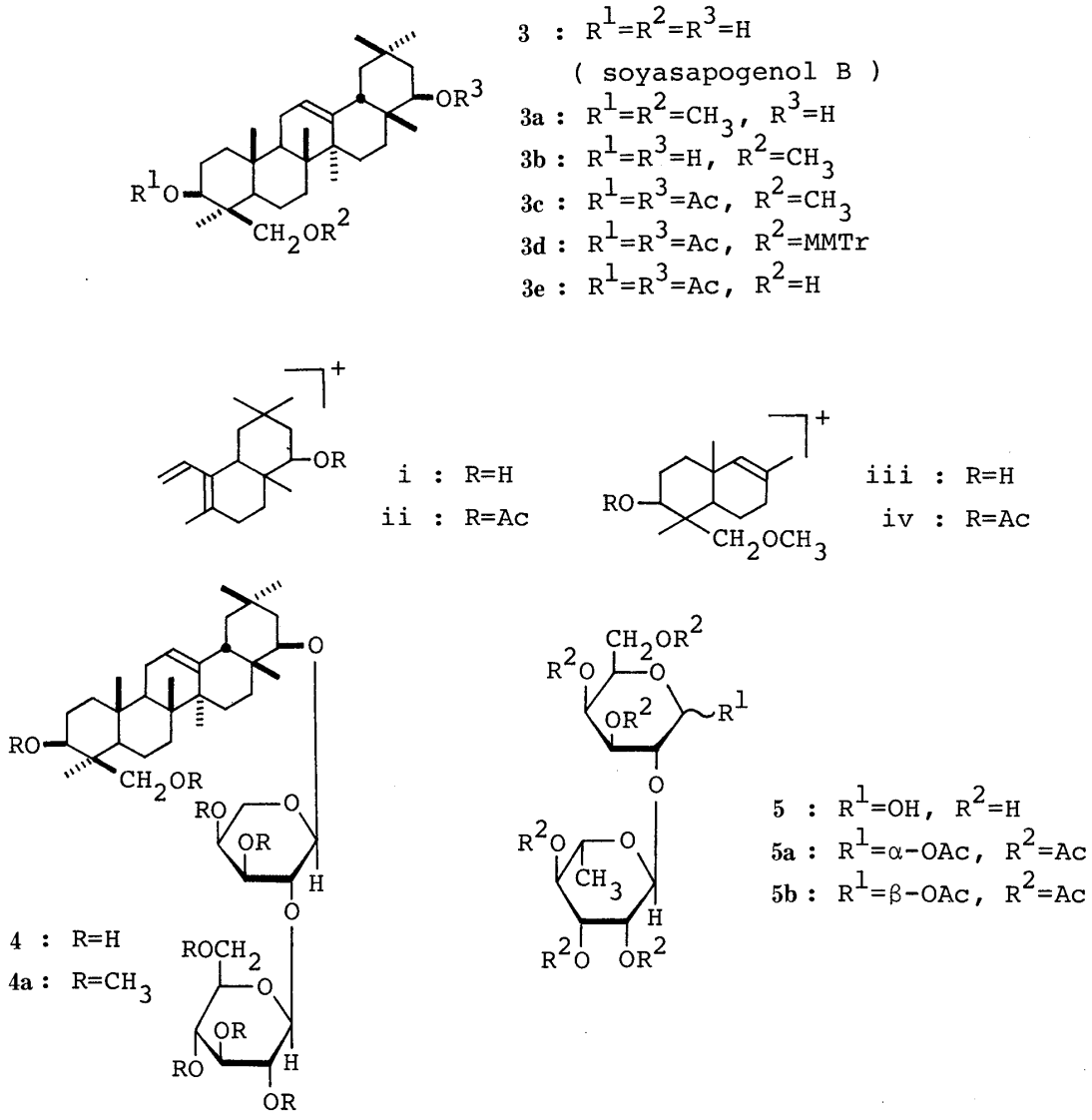


Chart 2

assignable to i and iii, which are derived through retro-Diels–Alder type fragmentation.[15] Acetylation of **3b** provided the diacetate (**3c**), which lacked hydroxyl absorption bands in its IR spectrum. The ^{1}H-NMR spectrum of **3c** exhibited signals due to two acetoxyl groups, one methoxyl group, and the 24-methyleneoxy function (as an AB quartet), together with signals assignable to 3α-H and 22α-H which are each geminal to an acetoxyl group. Retro-Diels–Alder type fragment ion peaks (ii, iv) were also observed in the MS of **3c**. Finally, treatment of soyasapogenol B (**3**) with *p*-anisylchlorodiphenylmethane (MMTrCl) followed by acetylation furnished **3d**, which, by mild acidic treatment, was converted to **3e**. Methylation of **3e** with methyl iodide and sodium hydride in tetrahydrofuran[16] followed by deacetylation afforded **3b**.

Based on the above-mentioned evidence, the structure of sophoraflavoside I has been determined as 3-*O*-[α-L-rhamnopyranosyl(1→2)-β-D-galactopyranosyl(1→2)-β-D-glucuronopyranosyl]-22-*O*-[β-D-glucopyranosyl(1→2)-α-L-arabinopyranosyl]soyasapogenol B (**2**). Sophoraflavoside I (**2**) seems to be the first example of a bisdesmoside of soyasapogenol B (**3**).

Soybean saponins [*e.g.*, soyasaponin I (**1**)] have been shown to exhibit various biological activities such as inhibitory effects on lipid oxidation and suppression of liver lesion generation in rats.[17] Taking these activities into consideration, the present characterization of

soyasaponin I (**1**) and sophoraflavoside I (**2**), which has a closely related structure to soyasaponin A_1,[1] seems to be quite suggestive for future investigations on the biological activity of Sophorae Radix.

Experimental

The instruments used to obtain physical data and the experimental conditions for chromatography were the same as described in our previous paper.[18]

Isolation of Soyasaponin I (1) and Sophoraflavoside I (2) from Sophorae Radix——Powdered Sophorae Radix (5 kg, imported from Korea, purchased from Tochimoto-Tenkaido, Osaka) was extracted with MeOH five times (10 l each, with heating under reflux for 5 h). Removal of the solvent from the combined MeOH solution under reduced pressure gave the MeOH extract (700 g). The MeOH extract was partitioned into 1-BuOH–water (1 : 1, 3 l) and removal of the solvent from the 1-BuOH phase under reduced pressure provided the 1-BuOH extract (400 g). The 1-BuOH extract was dissolved in MeOH (100 ml) and the solution was poured dropwise into AcOEt (1.5 l) with stirring. The precipitate (95 g) was collected by filtration and dried. Column chromatography of the precipitate over SiO_2 [2.5 kg, $CHCl_3$–MeOH–H_2O = 65 : 35 : 10 (lower phase)→6 : 4 : 1] and reversed-phase silica gel (Bondapak C_{18} 350 g, MeOH–H_2O = 2 : 3→1 : 1) followed by treatment with Dowex 50w × 8 (H^+ form) followed by recrystallization from aq. MeOH, furnished soyasaponin I (**1**, 4.4 g) and sophoraflavoside I (**2**, 6.6 g).[19]

Soyasaponin I (**1**) isolated here was shown to be identical with an authentic sample[6] by thin-layer chromatography (TLC) ($CHCl_3$–MeOH–H_2O = 65 : 35 : 10, lower phase; 1-BuOH–AcOEt–H_2O = 4 : 1 : 5, upper phase), mixed mp determination, and IR (KBr) spectral comparison. Furthermore, **1** (100 mg) was dissolved in MeOH (15 ml) and treated with ethereal diazomethane to afford **1a** (100 mg), which was shown to be identical with an authentic sample[6] by TLC (as described above for the identification of **1**), and IR (KBr) and ^{13}C-NMR (d_5-pyridine) spectral comparisons.

Sophoraflavoside I (**2**), mp 217—218 °C (colorless fine crystals), $[\alpha]_D^{20}$ −22° (c = 0.1, MeOH). *Anal.* Calcd for $C_{59}H_{96}O_{27} \cdot 3H_2O$: C, 54.87; H, 7.96. Found: C, 54.59; H, 8.10. IR ν_{max}^{KBr} cm^{-1}: 3400, 2920, 1714.

Acidic Hydrolysis of Sophoraflavoside I (2)——A solution of **2** (20 mg) in MeOH (5 ml) was treated with 20% aq. H_2SO_4 (5 ml) and the whole mixture was heated under reflux for 2 h. After removal of the MeOH under reduced pressure, the reaction mixture was extracted with AcOEt. The AcOEt extract was washed with sat. aq. $NaHCO_3$ and water, then dried over $MgSO_4$. The product obtained by evaporation of the solvent under reduced pressure was purified by preparative TLC ($CHCl_3$–MeOH = 20 : 1) to furnish soyasapogenol B (**3**, 6 mg). Soyasapogenol B (**3**) thus obtained was shown to be identical with an authentic sample[6] by TLC ($CHCl_3$–MeOH = 15 : 1, benzene–acetone = 3 : 1, *n*-hexane–acetone = 2 : 1) and IR (KBr) spectral comparison.

Methanolysis of Sophoraflavoside I (2)——A solution of **2** (20 mg) in 9% HCl–dry MeOH (1 ml) was heated under reflux for 1 h. The reaction mixture was neutralized with Ag_2CO_3 powder and the inorganic precipitate was removed by filtration. Concentration of the filtrate under reduced pressure yielded a suspension, from which the precipitate was collected by filtration. Crystallization of the precipitate from $CHCl_3$–MeOH furnished soyasapogenol B (**3**, 5 mg), which was identified as described above. The solvent was removed from the filtrate under reduced pressure to give a methyl glycoside mixture. The mixture was dissolved in pyridine (0.1 ml) and treated with *N,O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA, 0.2 ml) for 1 h. The product was then analyzed by gas-liquid chromatography (GLC) to identify the trimethylsilyl (TMS) derivatives of methyl arabinoside, methyl galactoside, methyl glucoside, methyl glucuronide, and methyl rhamnoside. The composition of these five methyl glycosides was determined from the GLC peak areas. GLC: 1) 1.5% silicone OV-1 on Chromosorb WAW DMCS (80—100 mesh), 3 mm × 1 m glass column; column temp. 150 °C; N_2 flow rate 30 ml/min; t_R, TMS-methyl arabinoside 3 min 23 s, 3 min 35 s, TMS-methyl galactoside 11 min 40 s, 13 min 20 s, 15 min 38 s, TMS-methyl glucoside 16 min 34 s, 19 min 50 s, TMS-methyl glucuronide 7 min 55 s, 18 min 20 s, TMS-methyl rhamnoside 4 min 01 s. 2) 1.5% silicone SE-30 on Chromosorb WAW DMCS (80—100 mesh), 3 mm × 1 m glass column; column temp. 150 °C; N_2 flow rate 30 ml/min; t_R, TMS-methyl arabinoside 3 min 05 s, 3 min 19 s, TMS-methyl galactoside 10 min 36 s, 12 min 08 s, 14 min 10 s, TMS-methyl glucoside 14 min 40 s, 17 min 53 s, TMS-methyl glucuronide 7 min 18 s, 16 min 34 s, TMS-methyl rhamnoside 3 min 42 s.

In another experiment, a solution of **2** (600 mg) in 9% HCl–dry MeOH (10 ml) was heated under reflux for 1 h. The product, obtained by treatment as described above, was subjected to column chromatography (SiO_2 20 g, $CHCl_3$–MeOH = 10 : 1) to furnish methyl arabinoside (35 mg). Methyl arabinoside thus obtained was hydrolyzed with 5% aq. HCl (reflux, 1 h). The reaction mixture was neutralized with Amberlite IRA-400 (OH^- form) and the resin was removed by filtration. After treatment with active charcoal (1 g), the water was removed from the filtrate under reduced pressure to furnish arabinose (11 mg), which was shown to be L form by its $[\alpha]_D^{25}$ value of +104° (c = 1.1, H_2O, 2 h after preparing the solution).

Sophoraflavoside I Methyl Ester (2a)——A solution of sophoraflavoside I (**2**, 100 mg) in MeOH (50 ml) was treated with excess ethereal diazomethane and the whole mixture was left standing at room temperature for 12 h. The

product, obtained by evaporation of the solvent under reduced pressure, was crystallized from MeOH to furnish **2a** (100 mg). **2a**, mp 223—225 °C (colorless fine crystals), $[\alpha]_D^{19}$ −18° ($c=0.5$, MeOH). *Anal.* Calcd for $C_{60}H_{98}O_{27}\cdot 3H_2O$: C, 55.20; H, 7.57. Found: C, 55.04; H, 7.78. IR ν_{max}^{KBr} cm^{-1}: 3440, 2930, 1735. ^{13}C-NMR: Table I.

Photolysis of Sophoraflavoside I (2)——A solution of **2** (440 mg) in MeOH (500 ml) in a Vycor tube was irradiated externally with a 500 W high-pressure mercury lamp (Eikosha, PIH-500) for 5 h while keeping the solution temperature below 20 °C. The reaction mixture was neutralized with 10% aq. K_2CO_3 and the solvent was evaporated off under reduced pressure. The product was partitioned into 1-BuOH–water (1 : 1). The product, obtained after removal of the solvent from the 1-BuOH phase under reduced pressure, was purified by column chromatography (SiO_2 20 g, $CHCl_3$–MeOH–H_2O = 20 : 3 : 1, lower phase) and crystallized from MeOH–acetone to furnish the prosapogenol (**4**). **4**, mp 105—106 °C (colorless fine crystals), $[\alpha]_D^{20}$ −14° ($c=0.2$, MeOH). *Anal.* Calcd for $C_{41}H_{68}O_{12}\cdot 2H_2O$: C, 62.41; H, 8.69. Found: C, 62.31; H, 8.90. IR ν_{max}^{KBr} cm^{-1}: 3404, 2904, 1079. The other product, obtained after removal of the solvent from the water phase, was acetylated with Ac_2O–pyridine (1 : 2, 3 ml) with stirring at room temperature for 12 h. The reaction mixture was poured into ice-water and the whole mixture was extracted with AcOEt. Work-up of the AcOEt extract in the usual manner gave the product, which was subjected to column chromatography (SiO_2 50 g, *n*-hexane–AcOEt = 2 : 1) to afford **5a** (30 mg) and **5b** (31 mg). **5a** and **5b** were shown to be identical with the corresponding authentic samples[13] by TLC (*n*-hexane–AcOEt = 2 : 3, *n*-hexane–acetone = 2 : 1, *n*-hexane–ether = 1 : 6) and IR ($CHCl_3$) spectral comparison.

Methanolysis of Prosapogenol (4)——A solution of **4** (5 mg) in 9% HCl–dry MeOH (1 ml) was heated under reflux for 1 h. The aglycone, which was obtained by work-up of the reaction mixture as described above for the methanolysis of **2**, was shown to be identical with soyasapogenol B (**3**) by TLC (as described above). The sugar portion was worked up and analyzed by GLC as the TMS derivatives, as described above for the methanolysis product of **2**, to identify methyl arabinoside and methyl glucoside in 1 : 1 ratio.

Methylation of Prosapogenol (4)——A solution of **4** (45 mg) in dimethyl sulfoxide (DMSO) (5 ml) was treated with dimsyl carbanion (3 ml)[6,14] and the whole mixture was stirred at 20 °C under a nitrogen atmosphere for 1 h. The reaction mixture was then treated with CH_3I (4 ml), stirred at room temperature in the dark for a further 3 h, and poured into ice-water. The whole mixture was extracted with AcOEt, and the AcOEt extract was washed with 10% aq. $Na_2S_2O_3$ and water, then dried over $MgSO_4$. Removal of the solvent under reduced pressure gave the product, which was purified by column chromatography (SiO_2 5 g, *n*-hexane–AcOEt = 2 : 1) and by crystallization from MeOH to furnish **4a** (25 mg). **4a**, mp 171—172 °C (colorless needles), $[\alpha]_D^{20}$ +18° ($c=0.78$, MeOH). *Anal.* Calcd for $C_{49}H_{84}O_{12}$: C, 68.02; H, 9.79. Found: C, 67.80; H, 10.02. IR $\nu_{max}^{CCl_4}$ cm^{-1}: 2919, 1099. ^{1}H-NMR ($CDCl_3$, δ): 0.80, 0.84, 0.91 (3H each), 0.90, 1.10 (6H each) (all s, *tert*-$CH_3\times 7$), 3.28, 3.36, 3.38, 3.39, 3.46, 3.50, 3.59, 3.69 (3H each, all s, $OCH_3\times 8$), 4.27 (1H, d, $J=7$ Hz, anom. H of glucosyl part), 4.88 (1H, br s, $W_{h/2}=5$ Hz, anom. H of arabinosyl part),[8c,20] 5.20 (1H, m, 12-H).

Methanolysis of 4a——A solution of **4a** (14 mg) in 9% HCl–dry MeOH (1 ml) was heated under reflux for 1 h. After cooling, the reaction mixture was worked up as described above for the methanolysis of **2**. The product, obtained as a precipitate, was crystallized from $CHCl_3$–MeOH to afford 3,24-di-*O*-methylsoyasapogenol B (**3a**, 6 mg), which was shown to be identical with an authentic sample[6b] by TLC (benzene–acetone = 4 : 1, *n*-hexane–acetone = 2 : 1, *n*-hexane–AcOEt = 1 : 1), mixed mp determination, and IR (CCl_4) and ^{1}H-NMR ($CDCl_3$) spectral comparisons. The other products, obtained from the filtrate, were identified as methyl 2,3,4,6-tetra-*O*-methylglucopyranoside (**a**) and methyl 3,4-di-*O*-methylarabinopyranoside (**b**) by TLC and GLC. TLC: benzene–acetone = 3 : 1, $CHCl_3$–MeOH = 15 : 2, *n*-hexane–AcOEt = 1 : 1. GLC: 3) 5% butane-1,4-diol succinate on Uniport B (80—100 mesh), 3 mm × 2 m glass column; column temp. 185 °C; N_2 flow rate 30 ml/min; t_R, **a** 4 min 12 s, 6 min 00 s, **b** 8 min 29 s, 16 min 39 s. 4) 15% polyneopentyl glycol succinate on Chromosorb WAW (80—100 mesh), 3 mm × 2 m glass column; column temp. 170 °C; N_2 flow rate 30 ml/min; t_R, **a** 6 min 36 s, 8 min 49 s, **b** 8 min 33 s, 10 min 12 s.

Methylation of Sophoraflavoside I (2)——A solution of **2** (107 mg) in DMSO (5 ml) was treated with dimsyl carbanion (5 ml) and the whole mixture was stirred at 20 °C under a nitrogen atmosphere for 2 h. The reaction mixture was then treated with CH_3I (5 ml) in the dark and stirred in a similar manner for 16 h. The reaction mixture was poured into ice-water and the whole was extracted with AcOEt. Work-up of the AcOEt extract as described above for methylation of **4** gave the product, which was purified by column chromatography (SiO_2 50 g, benzene–acetone = 8 : 1) followed by crystallization from MeOH to furnish **2b** (98 mg). **2b**, mp 162—163 °C (colorless needles), $[\alpha]_D^{20}$ −23.5° ($c=0.4$, MeOH). *Anal.* Calcd for $C_{75}H_{128}O_{27}$: C, 61.62; H, 8.83. Found: C, 61.33; H, 8.62. IR $\nu_{max}^{CCl_4}$ cm^{-1}: 2925, 1752, 1100. ^{1}H-NMR ($CDCl_3$, δ): 0.88, 0.91, 0.93 (3H each), 1.00 (6H), 1.07, 1.17 (3H each) (all s, *tert*-$CH_3\times 7$), 1.22 (3H, d, $J=4.5$ Hz, *sec*-CH_3), 3.27 (3H), 3.38 (9H), 3.39 (3H), 3.45 (12H), 3.47 (6H), 3.51, 3.58, 3.60, 3.65 (3H each) (all s, $OCH_3\times 15$), 3.78 (3H, s, $COOCH_3$), 4.27 (2H, d, $J=7$ Hz), 4.64 (1H, d, $J=7$ Hz) (anom. H × 3), 4.87 (1H, br s, $W_{h/2}=5$ Hz, anom. H of arabinosyl part),[8c,20] 5.07 (1H, m, 12-H), 5.22 (1H, br s, $W_{h/2}=4$ Hz, anom. H of rhamnosyl part). ^{1}H-NMR (C_6D_6, δ): 0.98 (3H), 1.11 (6H), 1.24 (9H), 1.46 (3H) (all s, *tert*-$CH_3\times 7$), 1.46 (3H, d, $J=5$ Hz, *sec*-CH_3), 3.06 (3H), 3.20 (6H), 3.29, 3.35 (3H each), 3.39 (6H), 3.40 (3H), 3.42 (12H), 3.51, 3.54, 3.58 (3H each) (all s, $OCH_3\times 15$), 3.95 (3H, s, $COOCH_3$), 4.35, 4.46, 4.91 (1H each, all d, $J=7$ Hz, anom. H × 3), 5.13 (1H, br s, $W_{h/2}=5$ Hz, anom. H of arabinosyl part),[8c,20] 5.31 (1H, m, 12-H), 5.53 (1H, br s, $W_{h/2}=4$ Hz, anom. H of rhamnosyl part).

$LiAlH_4$ Reduction of 2b Followed by Methanolysis——A solution of **2b** (50 mg) in dry ether (5 ml) was treated with a suspension of $LiAlH_4$ (30 mg) in dry ether (2 ml) and the mixture was stirred at 20 °C for 1 h. After quenching of the reaction with wet ether, the reaction mixture was made weakly acidic with 5% aq. H_2SO_4 and the whole was extracted with AcOEt. Work-up of the AcOEt extract in the usual manner gave the reduction product (45 mg). The product was dissolved in 9% HCl–dry MeOH (2 ml) and the solution was heated under reflux for 1 h. After cooling, the reaction mixture was neutralized and worked up as described above for the methanolysis of **2**. The product, obtained as a precipitate, was crystallized from $CHCl_3$–MeOH to furnish **3b** (15 mg). **3b**, mp 190—191 °C (colorless needles), $[\alpha]_D^{20}$ +50.1° ($c=1.0$, $CHCl_3$). High-resolution MS: Found 472.390. Calcd for $C_{31}H_{52}O_3$ (M^+) 472.389. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3450, 2916, 1100. ^{1}H-NMR ($CDCl_3$) δ: 0.86 (3H), 0.93 (6H), 0.95, 1.03, 1.11, 1.21 (3H each) (all s, *tert*-$CH_3\times7$), 3.30 (3H, s, OCH_3), 3.20, 3.88 (2H, ABq, $J=9$ Hz, 24-H_2), 5.23 (1H, t-like). MS *m/z* (%): 472 (M^+ <1), 234 (i, 100), 238 (iii, 10), 216 (i-H_2O, 74). The other products, obtained from the filtrate, were identified as **a**, **b**, methyl 2,3,4-tri-*O*-methylrhamnopyranoside (**c**), methyl 3,4,6-tri-*O*-methylgalactopyranoside (**d**), and methyl 3,4-di-*O*-methylglucopyranoside (**e**) by TLC and GLC as described above for the methanolysis product of **4a**. GLC: 3) t_R, **a**, **b** the same as in the case of **4a**, **c** 3 min 05 s, 4 min 14 s, **d** 9 min 11 s, 14 min 00 s, **e** 17 min 20 s, 20 min 03 s. 4) t_R, **a**, **b** the same as in the case of **4a**, **c** 3 min 30 s, 4 min 44 s, **d** 21 min 14 s, 31 min 42 s, **e** 37 min 09 s, 42 min 54 s.

Acetylation of 3b——A solution of **3b** (20 mg) in pyridine (2 ml) was treated with Ac_2O (2 ml) and the mixture was stirred at 20 °C for 12 h. The reaction mixture was poured into ice-water and the whole was extracted with AcOEt. Work-up of the AcOEt extract in the usual manner gave the product, which was crystallized from $CHCl_3$–MeOH to furnish **3c** (20 mg). **3c**, mp 224—225 °C (colorless needles), $[\alpha]_D^{20}$ +40.8° ($c=1.0$, $CHCl_3$), High-resolution MS: Found: 556.413. Calcd for $C_{35}H_{56}O_5$ (M^+) 556.413. IR $\nu_{max}^{CCl_4}$ cm^{-1}: 2950, 1730, 1244. ^{1}H-NMR ($CDCl_3$) δ: 0.82, 0.90, 0.98 (3H each), 1.00 (9H), 1.13 (3H) (all s, *tert*-$CH_3\times7$), 2.00, 2.02 (3H each, both s, OAc×2), 3.27 (3H, s, OCH_3), 3.34, 3.36 (2H, ABq, $J=10$ Hz, 24-H_2), 4.51 (1H, t-like, 3-H), 4.61 (1H, t-like 22-H), 5.22 (1H, t-like, 12-H). MS *m/z* (%): 556 (M^+, 2), 276 (ii, 13), 216 (ii-AcOH, 100), 280 (iv, 7).

Monomethoxytritylation of 3 Followed by Acetylation——A solution of **3** (500 mg) in pyridine (10 ml) was treated with MMTrCl (1.0 g) and the whole mixture was stirred at 20 °C for 1 h. The reaction mixture was then treated with Ac_2O (0.5 ml) and stirred at 20 °C for a further 20 h. The reaction mixture was poured into ice-water and the precipitated product was collected by filtration. Purification of the product by column chromatography (SiO_2 50 g, $CHCl_3$) furnished **3d** (608 mg). **3d**, white powder, $[\alpha]_D^{20}$ +60.6° ($c=0.9$, $CHCl_3$). *Anal.* Calcd for $C_{54}H_{70}O_8$: C, 76.56; H, 8.33. Found: C, 76.26; H, 8.27. IR $\nu_{max}^{CCl_4}$ cm^{-1}: 2955, 1730, 1250. ^{1}H-NMR ($CDCl_3$) δ[21]: 0.47, 0.80, 0.84, 0.89, 0.99, 1.11, 1.16 (3H each, all s, *tert*-$CH_3\times7$), 2.00 (6H, s, OAc×2), 3.07, 3.37 (2H, ABq, $J=10$ Hz, 24-H_2), 3.76 (3H, s, OCH_3), 4.61 (2H, m, 3,22-H), 5.17 (1H, m, 12-H), 6.70—7.50 (14H, m, arom. H).

Removal of the MMTr Group from 3d——A solution of **3d** (100 mg) in tetrahydrofuran (THF) (20 ml) was treated with BF_3–ether (20 ml) and the whole mixture was stirred at 20 °C for 24 h. The reaction mixture was poured into ice-water and the whole was extracted with AcOEt. Work-up of the AcOEt in the usual manner gave the product, which was purified by column chromatography (SiO_2 12 g, $CHCl_3$) followed by crystallization from $CHCl_3$–MeOH to furnish **3e** (50 mg). **3e**, mp 207—209 °C (colorless needles), $[\alpha]_D^{20}$ +41.9° ($c=0.9$, $CHCl_3$). High-resolution MS: Found 542.394. Calcd for $C_{34}H_{54}O_3$ (M^+) 542.392. IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3450, 1730, 1250. ^{1}H-NMR ($CDCl_3$) δ: 0.82, 0.90, 0.93, 0.97, 1.01, 1.09, 1.15 (3H each, all s, *tert*-$CH_3\times7$), 2.01, 2.06 (3H each, both s, OAc×2), 3.40, 4.12 (2H, ABq, $J=12$ Hz, 24-H_2), 4.63 (2H, m, 3,22-H), 5.24 (1H, t-like, 12-H). MS *m/z* (%): 542 (M^+, 19), 276 (ii, 15), 216 (ii-AcOH, 100).

Methylation of 3e Followed by Deacetylation——A solution of **3e** (50 mg) in THF (10 ml) was treated with CH_3I (1 ml) and NaH (100 mg, defatted with dry ether before use). The reaction mixture was stirred at 17 °C for 10 h, then poured into ice-water, and the whole was extracted with AcOEt. Work-up of the AcOEt extract in the usual manner gave the product, which was purified by preparative TLC ($CHCl_3$–MeOH = 150 : 1) to furnish **3c** (26 mg). A solution of **3c** (20 mg) in MeOH (1 ml) was treated with 10% NaOMe–MeOH (1 ml) and the reaction mixture was stirred at 20 °C for 24 h, neutralized with 5% aq. HCl, and then extracted with $CHCl_3$. The $CHCl_3$ extract was washed with aq. sat. $NaHCO_3$ and water. Removal of the solvent from the $CHCl_3$ extract under reduced pressure gave the product, which was crystallized from $CHCl_3$–MeOH to furnish **3b** (15 mg). **3b** obtained here was shown to be identical with that (**3b**) obtained above from **2** by TLC ($CHCl_3$–MeOH = 50 : 1, *n*-hexane–AcOEt = 1 : 1, benzene–acetone = 4 : 1), mixed mp determination, and IR ($CHCl_3$) and ^{1}H-NMR ($CDCl_3$) spectral comparisons.

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